UNITED STATES ARMY ENVIRONMENTAL HYGIENE AGENCY

ABERDEEN PROVING GROUND, MD 21010-5422

PHASE 1
PRELIMINARY ASSESSMENT OF THE RELATIVE TOXICITY
OF TETRAGLYCINE HYDROPERIODIDE
STUDY NO. 75-51-0742-91
JANUARY 1988 - AUGUST 1991

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19 ABSTRACT (Continue on reverse if necessary and identify by block number) A Preliminary Assessment of the Relative Toxicity of Technical Grade Tetraglycine Hydroperiodide (TGHP), the active ingredient in a military water purification tablet (MIL-W-283) has been completed. TGHP is moderately toxic by ingestion when prepared as a aqueous slurry. The compound has no potential for causing sensitization. However, TGHP did produce mild but reversible primary skin irritation, and the technical grade powder did cause severe and nonreversible injury to the eye. TGHP did not exhibit mutagenic activity in three of the four mutagenicity assays performed. In the Chromosome Aberration Assay, TGHP did produce a significant increase in chromosomal aberrations in CHO cells in the presence, but not in the absence, of metabolic activation. This single positive finding in the Chromosome Aberration Assay, in the presence of metabolic activation, is not considered sufficient to classify TGHP a potential human-cell mutagen. Recommend conducting further toxicological studies with tetraglycine hydroperiodide in support of the requirement to provide data to the U.S. Environmental Protection Agency for the reregistration of the Army's Water Purification Tablets.

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DEPARTMENT OF THE ARMY
U. S. ARMY ENVIRONMENTAL HYGIENE AGENCY
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REPLY TO ATTENTION OF

EXECUTIVE SUMMARY
PHASE 1
PRELIMINARY ASSESSMENT OF THE RELATIVE TOXICITY OF TETRAGLYCINE HYDROPERIODIDE
STUDY NO. 75-51-0742-91
JANUARY 1988 - AUGUST 1991

1. A Preliminary Assessment of the Relative Toxicity of Technical Grade Tetraglycine Hydroperiodide (TGHP), the active ingredient in a military water purification tablet (MIL-W-283) was completed in August of 1991. Report is enclosed.

2. ESSENTIAL FINDINGS. TGHP is moderately toxic by ingestion when prepared as a aqueous slurry. The compound has no potential for causing sensitization. However, TGHP did produce mild but reversible primary skin irritation, and the technical grade powder did cause severe and nonreversible injury to the eye. The TGHP did not exhibit mutagenic activity in three of the four mutagenicity assays performed. In the Chromosome Aberration Assay, TGHP did produce a significant increase in chromosomal aberrations in CHO cells in the presence, but not in the absence, of metabolic activation. This single positive finding in the Chromosome Aberration Assay, in the presence of metabolic activation, is not considered sufficient to classify TGHP a potential human-cell mutagen.

3. RECOMMENDATIONS. Recommend conducting further toxicological studies with tetraglycine hydroperiodide in support of the requirement to provide data to the U.S. Environmental Protection Agency for the reregistration of the Army's Water Purification Tablets.

DEPARTMENT OF THE ARMY
U. S. ARMY ENVIRONMENTAL HYGIENE AGENCY
ABERDEEN PROVING GROUND, MARYLAND 21010-5422

REPLY TO ATTENTION OF
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PHASE 1
PRELIMINARY ASSESSMENT OF THE RELATIVE TOXICITY OF TETRAGLYCINE HYDROPERIODIDE
STUDY NO. 75-51-0742-91
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1. REFERENCES. See Appendix A for a list of references.

2. AUTHORITY.

a. Memorandum, HSC, HSCL-P, 26 August 1987, subject: Toxicity Clearance.

b. Memorandum, TROSCOM, STRNC-YEP, 29 July 1987, subject: Toxicity Clearance For Tetraglycine Hydroperiodide, Active Ingredient in Water Purification Tablets, Iodine (MIL-W-283).

3. PURPOSE. The objective of these studies was to determine the relative toxicity of Tetraglycine Hydroperiodide (TGHP), the active ingredient in a military water purification tablet (MIL-W-283). This information will be used to provide guidance for future acute and subchronic toxicity studies with TGHP to comply with the U.S. Environmental Protection Agency's (EPA) data call-in (reference 1) for toxicological data on antimicrobial pesticide active ingredients.

4. BACKGROUND.

a. Tetraglycine hydroperiodide (formerly triglycine hydroperiodide) is a periodide that is more stable than most other water purification compounds. It is produced by reacting elemental iodine, potassium iodide, glycine and hydrochloric acid (reference 2). A tablet containing tetraglycine hydroperiodide, sodium acid phosphate, and a small amount of talc was developed.

b. The U.S. Army Natick Research, Development and Engineering Center now holds Registration No. 40510-3 with the EPA for Iodine Water Purification Tablets (reference 3). The active ingredient in these tablets, TGHP, is an organic iodine-liberating compound. The "iodine, made available in low and well-controlled concentrations, is effective in the disinfection of drinking water" (references 4, 5, 6, 7, 8 and 9).

Use of trademarked names does not imply endorsement by the U.S. Army but is intended to assist in identification of a specific product.

c. The EPA has issued a data call-in (reference 1) for subchronic and chronic toxicological data for antimicrobial pesticide active ingredients such as TGHP.

d. This Agency was tasked by the U.S. Army Health Services Command to conduct the appropriate studies required to support the reregistration of the Army's EPA Registration No. 40510-3 for Water Purification Tablets. Previous toxicity studies reviewed by Oscar Hunter Adams (reference 10) to initially register these tablets in 1951 were conducted with a soluble iodine compound, sodium iodide, to yield concentrations equivalent to, or more than, those used in the field purification process. Tetraglycine hydroperiodide, the active ingredient was not used in these studies because of its high cost. Also, its low solubility would not permit the desired concentration to be achieved with conventional feeding equipment.

e. Water Purification Tablets containing TGHP are commercially available under the trade names Globaline, Potable Aqua and Coughlan's. One tablet of any of these TGHP-containing preparations will provide 8 ppm iodine when dissolved in one liter of water. The composition of the Globaline tablet is: TGHP, 19.1-21.3 mg; disodium dihydrogen pyrophosphate (an acidic excipient), 82.5-92.3 mg; and talc, <6 mg (reference 5).

5. GENERAL. The collection of laboratory data, statistical analysis and report preparation was also performed by SSG Rodney M. Cantu who has since departed from this Agency.

6. MATERIALS.

a. Test Compound.

(1) The test compound was technical grade TGHP, a black powder with a brassy-bronze metallic luster in reflected light. The TGHP has a CAS Registry Number of 7097-60-1, a molecular formula of $C_{10}H_{42}I_7N_5O_{10}$, and a molecular weight of 1490.95. The solubility in water is listed as 380 g/l at 25 °C (reference 11). All technical grade TGHP was supplied by Wisconsin Pharmacal Company, Jackson, Wisconsin.

(2) A Certificate of Analysis (reference 12) from Wisconsin Pharmacal indicated that the 25 pounds of TGHP (lot number 10704, formula 4018) shipped for toxicity testing passed their quality control analytical analysis with 40.63 mg of Titratable Iodine (T.I.). The acceptable range was 39.5 to 42.6 mg T.I. The TGHP provided is the same used in their product (EPA Reg. No. 305-37) and in the Army's product (EPA Reg. No. 40510-3) which Wisconsin Pharmacal Company manufactures. The molecular chemical formula of TGHP is represented below:

$$4[(NH_2CH_2COOH)_2HI] \cdot 5I_2$$

b. Animals.*†

(1) Ocular and dermal evaluations, performed at USAEHA with TGHP, were conducted using New Zealand white rabbits from Dutchland Laboratory, Denver, Pennsylvania. Male, Albino-Hartley guinea pigs, also from Dutchland Laboratories, were used for sensitization studies. Sprague-Dawley, Wistar-derived rats from Charles River Breeding Laboratories, Inc., Wilmington, MA, were used for determination of acute oral toxicities. All animals were housed individually in wire-bottom stainless steel cages. All animals were maintained on commercial chow (Purina® Rabbit Chow 5322, Purina Guinea Pig Ration 5026 and Purina Certified Rodent Chow 5026) and water ad libitum. Ambient conditions were 24 °C ± 2 °C, 40-60 percent relative humidity and a 12-hour light dark sequence. All studies were conducted in accordance with current standing operating procedures and Federal regulatory guidelines (references 13 and 14). Statements of technical compliance and analytical quality assurance appear as Appendices B and C.

(2) The results from the animal toxicity studies were categorized using the toxicity category table published in 40 CFR 162 and are described in Table 1.

7. METHODS.

a. Primary Skin Irritation Studies. The test for acute primary skin irritation was performed to evaluate the potential for local toxic effects of chemicals expected to come in contact with the skin. It consists of one period of topical application for 24 hours (the exposure period) and an observation period of 7 days. In this study, the irritant response from a paste of 0.5 gm of the test material and H_2O was evaluated following a single 24-hour occluded application to three intact and three abraded skin sites of six New Zealand white rabbits (2.0 to 3.5 Kg). The Draize (reference 15) scoring system (Appendix D) was used for the evaluation of skin reactions.

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* In conducting the studies described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals", U.S. Department of Health and Human Services, NIH Publication No. 85-23, 1985.

† The studies reported herein were performed in animal facilities fully accredited by the American Association for the Accreditation of Laboratory Animal Care.

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TABLE 1. EPA HAZARD INDICATORS

Hazards Indicators	Toxicity Categories I	II	III	IV
Oral LD_{50}	Up to and including 50 mg/kg	From 50 thru 500 mg/kg	From 500 thru 5000 mg/kg	Greater than 5000 mg/kg
Skin Effects	Corrosive	Severe irritation at 72 hours	Moderate irritation at 72 hours	Mild or slight irritation at 72 hours
Eye Effects	Corrosive corneal opacity not reversible within 7 days.	Corneal opacity reversible within 7 days; irritation persisting for 7 days.	No corneal opacity; irritation reversible within 7 days.	No irritation

b. Eye Irritation. An eye-irritation study was performed by administering a single 0.1 gm dose of technical grade chemical powder to one eye of each of six New Zealand white rabbits (2.0 to 3.5 Kg). The eyelids were held open momentarily and then released gently and the animal allowed to blink freely. Any solid on the fur surrounding the eye was wiped off gently with tissue paper. After 24 hours the material was washed out of the eyes of the six rabbits on test. The opposite eye was left untreated and served as a control. Eyes were examined for gross signs of irritation at 24, 48, and 72 hours, and 7, 14 and 21 days following application. Scoring of irritation effects was based on the Draize method in which the total score for the eye is the sum of all scores obtained for the cornea, iris, and conjunctiva. No gross pathology or histopathology was done. Categorizing of the responses was based on the 24-hour evaluation.

c. Sensitization.

(1) Sensitization studies were performed to determine the potential of the test material for causing sensitization reactions following dermal applications. Female albino Hartley guinea pigs weighing between 375 and 425 gms were used for all tests.

(2) This test procedure was based on the studies of Buehler (reference 16) and used to predict the possible delayed contact hypersensitivity to a chemical. Technical grade test compound in 0.3 ml aliquots of 0.1 percent in 80 percent ethanol, was applied on Webril® patches to the shaved flanks of 10 guinea pigs. Treatment was for 6 hours, once per week, for 3 weeks. Challenge doses followed a 2-week rest period and included 10 previously untreated control animals. The skin responses were scored at 24- and 48-hour post challenge by the Draize method of scoring. All animals were depilated with NEET® cream, thoroughly washed with warm water and then towel dried 3 hours prior to scoring.

(3) A positive control group received dinitrochlorobenzene (DNCB). Induction was 0.1 percent DNCB (w/v) in 80 percent ethanol and the challenge dose was 0.01 percent (w/v) in 80 percent ethanol.

d. Acute Oral Studies. Acute toxicity studies were performed to determine the adverse effects occurring from a single dose of a substance. This type of study identifies the relative toxicity of a compound, investigates its mode of action and specific toxic effects, and determines the existence of species differences. The most frequently used acute toxicity test involves determination of the median lethal dose (LD_{50}) of the compound. The LD_{50} is defined as a statistically derived expression of a single dose of a material. In the present study, single doses of TGHP was administered as a 100 mg/ml aqueous slurry to male rats. The TGHP was prepared in this manner due to its' low solubility in water and its' tendency to sublime upon heating or extended mixing. The test material was administered by gavage to mature rats via stainless steel stomach tubes. A 14-day observation period was used to observe death or clinical signs. Animals were weighed at 1, 3, 7, and 14 days after exposure. All survivors were euthanized at 14 days and submitted for gross necropsy. Calculations of the LD_{50} were performed by the Probit method as described by Finney (reference 17).

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® NEET is a registered tradename of Whitehall Laboratories, New York, New York.

e. Salmonella Typhimurium/Microsome Reverse Mutation Assay: Plate Incorporation Method [Integrated Laboratory Systems (ILS) Project No. ILS R031].† The object of this study was to evaluate TGHP for genetic activity in microbial assays with and without the addition of mammalian metabolic activation. The Plate Incorporation method was used with five strains of Salmonella typhimurium (TA1535, TA100, TA1537, TA1538 and TA98) in evaluating mutagenic potential. Mutagenic activity of each dose level of TGHP tested in a specific tester strain was evaluated as the ratio of the mean (the average of duplicate plates) number of revertant colonies to the mean number of colonies appearing on solvent control plates, or:

$$\text{Mutagenic Index} = \frac{\text{Induced Revertants}}{\text{Spontaneous Revertants}}$$

A test article is regarded as a mutagen if it produces a dose-dependent increase in the mutagenic index, or if it consistently produces a mutagenic index of 2.0 (3.0 for strains of TA1535, TA1537, and TA1538) or higher. The TGHP was tested directly and in the presence of liver homogenates (S-9 fraction) from rats treated with Aroclor® 1254. Concurrent positive and negative controls were run along with five dose points of the diluent in preparing stock solutions. All tests were run in triplicate plates. The high dose was limited to 1 mg per plate for the S-9 activated portion and 0.1 mg for the nonactivated portion with five dose points separated by one half log intervals.

f. Chromosomal Aberrations Assay$ (ILS Project No. ILS R031). The objective of this *in vitro* assay was to test the mutagenic potential of TGHP and/or its metabolites as measured by its ability to induce structural chromosomal aberrations in Chinese hamster ovary (CHO) cells, with and without metabolic activation. Metabolic activation was incorporated into the assay

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† Work performed under contract by Integrated Laboratory Systems, Research Triangle Park, North Carolina (ILS Contract No. DAAD05-88-C-0073).

$ Work performed under contract by Integrated Laboratory Systems, Research Triangle Park, North Carolina (ILS Contract No. DAAD05-88-C-0073).

®Aroclor is a registered tradename of Monsanto Chemical Company, St. Louis, Missouri.

by the addition of S-9, the post mitochondrial supernatant fraction of liver homogenates, obtained from Sprague Dawley rats induced with Aroclor 1254. The S-9 was purchased from Molecular Toxicology, Rockville, Maryland and stored a -70 °C. The CHO-K1 used in this assay were a proline auxotroph with a modal chromosome number of 20, a population doubling time of 10-12 hours, and cloning efficiency of approximately 20 percent. The CHO cells were obtained from Environmental Health Research and Testing, Durham, North Carolina. Selection of dose levels was based upon toxicity as indicated by the loss of growth potential of the cells. Cells seeded 16-24 hours earlier were exposed to 1000, 500, 100, 50 and 10 µg/mL of TGHP for 26 hours at 37 °C in the absence of S-9 and for 2 hours in the presence of S-9. The compound 5-bromo-2'-deoxyuridine was added to all cultures 2 hours after initiation of treatment. Following a 24-hour post-treatment growth period, with colcemid present for the last 2 hours, the cells were harvested by either trypsination or scraping with a teflon scraper. The cells were exposed to a hypotonic solution and then fixed using 3:1 methanol:glacial acetic acid. Mitomycin C at 1.0 µg/mL was used as the positive control in the nonactivated portion. Cyclophosphamide at 25 µg/Ml was used as the positive control in the S-9 activated portion. In the actual nonactivated study, duplicate cultures were exposed to 800, 600, 300, 60 and 30 µg/mL of TGHP for 8 hours. Following the exposure period, colcemid was added to all cultures for an additional 2 hours. In the S-9 activated study, cells were exposed to 600, 300, 60, 30 and 3.0 µg/mL of TGHP for 2 hours, washed and incubated for another 8 hours with colcemid present for the last 2 hours. After treatment with hypotonic solution, the cells were fixed as described in the dose range finding procedure. A minimum of 100 metaphase cells from each dose level with 20 ± 2 centromeres were examined. The mutagenic potential of the test agent was measured by its ability to increase structural chromosomal aberrations in a dose-responsive manner when compared to a control group.

g. Mouse Lymphoma Mutation Assay** (ILS Project No. ILS R031). The mouse lymphoma mutagenesis assay was used to evaluate the mutagenic potential of TGHP or its metabolites. The object of this assay was to determine the ability of TGHP to induce mutations at the thymidine kinase locus as assayed by colony growth of L5178Y TK ± mouse lymphoma cells. The TGHP was examined with and without exogenous metabolic activation by Aroclor induced rat liver microsomes. Based on the data derived

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** Work performed under contract by Integrated Laboratory Systems Research Triangle Park, North Carolina (ILS Contract No. DDAD05-88-C-0073).

from toxicity tests, TGHP was diluted and serial dilutions were carried out to produce the dosing solutions for the nonactivated and activated portions of the study. The nonactivated cultures successfully cloned were treated with eight doses of 36 to 4.8 µg/mL and the S-9 activated culture clones were treated with eight doses of 1001 to 134 µg/mL. The positive control articles were ethyl methanesulfonate at 0.50 and 0.25 µg/mL for nonactivation and 7,12 dimethylbenz(a)anthracene at 2.5 and 4.0 µg/mL for assays performed with activation. A solvent control was included with both types assay. Cell cultures were exposed to treatment, washed, cloned, and incubated before colony counts were made. The measurement of the toxicity of each treatment was the relative suspension growth of the cells over a 2-day expression period multiplied by the cloning efficiency (determined by colony counts), relative to the average solvent control. The following criteria was used as a guideline in judging the significance of the activity of TGHP in this system.

(1) Positive. If there is a positive dose response and one or more of the doses in the 10 percent or greater total growth range exhibit a mutant frequency that is two-fold greater than the background level.

(2) Equivocal. If there is no dose response but any one or more of the three highest doses with 10 percent or greater total growth exhibit a two-fold increase in mutant frequency over background, or if there is a dose response but no culture exhibits, a two-fold increase in mutant frequency over background.

(3) Negative. If there is no dose response in cultures with 10 percent or greater total growth and none of these test cultures exhibit a two-fold or greater increase in mutant frequency over background.

h. Unscheduled DNA Synthesis Assay‡‡ (SRI Study No. 7593-B01-89, ILS Study No. RO31-1AD). The objective of this assay was to detect DNA damage caused by TGHP, or an active metabolite, by measuring unscheduled DNA synthesis (UNA) in primary rat hepatocytes in vitro. The indicator cells for this assay were hepatocytes obtained from adult male Fisher 344 rats received by

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‡‡ Work performed under contract for Integrated Laboratory Systems, Research Triangle Park, North Carolina by SRI International, Menlo Park, California (SRI Project No. LSC-7593).

SRI Laboratory Animal Department from the Indianapolis, Indiana, facility of Harlan Sprague-Dawley Laboratories, Inc. The USD assay was initiated with a series of 10 concentrations ranging from 0.05 to 1,000 µg/mL. Dimethyl sulfoxide (DMSO, 1 percent) was chosen as the negative control/solvent for the test solution. The positive control for this assay was 2-acetylaminofluorene (2-AAF, 3.0 µg/mL). The following criteria was used as a guideline in judging the significance of the activity of TGHP in this system. Frequency distributions of grain counts for each test concentration and the average and median grain counts will be calculated and compared with control values.

(1) Positive. The TGHP will be considered unequivocally positive if the mean net grain count for any dose group is greater than 5 grains/nucleus (N/G).

(2) Negative. The TGHP will be considered unequivocally negative if the mean net grain count for all dose groups is less than 0 NG and the percentage of cells in repair (percent IR) is less than 10 percent.

(3) Other. When results fall within 0-5 NG, or when the percent IR exceeds 10 percent, the presence of a dose response, the frequency distribution of cellular responses, increases in the percentage of cells in repair, and reproducibility of data among animals will all be considered and the test article will then be classified as "negative," "weak positive," or "equivocal."

8. RESULTS.

a. Primary Skin Irritation Studies. The potential for primary skin irritation was tested by a 24-hour application of the test material to the intact and abraded skin of six rabbits. The TGHP, under the described test conditions, produced intense staining of the application site causing difficulty in reading irritation scores 24 hours after application. Mild irritation of intact and abraded skin was observed at 72 hours. It also caused very slight to slight edema at 24 and 72 hours on intact and abraded skin. All edema and erythema was absent 7 days post application. The USAEHA category of II was assigned to these responses (Appendix E). The EPA hazard indicator index places these skin responses in grade IV (Table 1). Appendix D and E describe the scale (Draize System) for scoring skin lesions and define categories of skin effects. Results from each application are shown in detail in Appendix F.

b. Eye Irritation. The potential of TGHP to cause ocular damage was studied by the instillation of TGHP powder as described to one eye of each of six rabbits. Due to the corrosive action of the test material a local anesthetic was applied to the exposed eyes. Neovascularization (pannus tenuis) was observed in the exposed eye of three of the six rabbits 14 days after the application of TGHP. The grayish membrane-like vascularized tissue with few blood vessels and slight opacity covered approximately one quarter of the upper eye. The TGHP was corrosive to rabbit eyes, causing severe injury to the conjunctiva and cornea that persisted through 21 days. The USAEHA category of F was assigned to these responses (Appendix H). The EPA Eye Effect Category ratings were calculated to be I (as shown in Table 1). Appendices G and H describe the scale for scoring ocular lesions and define categories of eye effects. Results from each application are shown in detail in Appendix I.

c. Sensitization. The challenge dose of TGHP, after a 2-week rest period, did not produce irritation scores greater than those for the initial application. By contrast, positive control scores were greatly increased at challenge when compared to initial scores. These findings indicate a lack of sensitizing potential for TGHP at the concentrations tested.

d. Acute Oral Studies. A tabular presentation of the median lethal values in male rats of technical grade TGHP follows:

TABLE 2. TGHP, ACUTE LETHAL STUDIES IN MALE RATS

LD_{50} mg/kg	95% Confidence Interval mg/kg	Slope (+- SE)	Signs
838	736-952	4.82 (3.43)	Prostration and bloody nasal discharge at all lethal dosages; necrotic tail tips sporadic occurrence in dose groups above 500 mg/kg.

NOTE: Red swollen tail tips also observed in two control rats.

Under the test conditions of this study (paragraph 7d) TGHP is classified as an EPA Category III material (Table 1).

e. Mutagenicity Plate Assay. (Integrated Laboratory Systems (ILS) Project No. ILS R301). A toxicity test was conducted on TGHP using strain TA100 at 10, 1.0, and 0.1 mg/plate. All three doses were toxic in the nonactivated half and only the 10 mg/plate dose was toxic with S-9 activation. The highest dose was set at 1.0 mg for the S-9 activated portion and 0.1 mg for the non-activated portion. The top dose of the nonactivated portion of TA98 and TA100 was lowered to 50 µg/plate after an initial test in those strains showed increased toxicity at 100 µg/plate. The mutagenicity assays were conducted at five dose levels from 1 µg to 100 µg (TA1535, TA1537 & TA1538) and from .5 µg to 50 µg (TA98 & TA100) without activation. The five dose levels extended from 10 µg to 1000 µg with activation. All tests were done using three plates per dosage level. The results of the assay indicated a lack of mutagenic activity in all five strains both with and without S-9 activation.

f. Chromosomal Aberration Frequency Assay.

(1) Range Finding Assays. Over the dose range tested, treatment with TGHP at 1000 µg/mL resulted in a lack of growth in both activated and nonactivated cultures. Between 10 and 500 µg/mL nonactivated cultures exhibited a dose-dependent decrease in mitotic index but no alteration in the replicative index. For activated cultures, neither the mitotic index nor the replicative index were significantly altered. The decline in mitotic index concomitant with no change in the replicative index suggests that the decline in the proportion of proliferating cells was not due to inhibition of cell cycle progression. Based on these data, the maximum dose to be tested in both activated and nonactivated cultures was selected to be 600 µg/mL.

(2) Chromosomal Aberrations Assay Without Metabolic Activation. Treatment with TGHP in CHO cells over the test range (30 to 600 g/mL), did not induce a significant increase in the percentage of metaphase cells containing at least one chromosomal aberration. The positive control, MMC at 1 g/mL, was clastogenic.

(3) Chromosomal Aberrations Assay With Metabolic Activation. Based on 100 metaphase cells per dose group, treatment with TGHP appeared to induce a significant increase in clastogenic damage. Additional cells were scored under code and the data combined. Under these conditions, treatment with TGHP induced a small but significant increase in the percentage of metaphase cells containing at least one chromosomal aberration. A statistical comparison of individual dose data at all treatment doses with the concurrent control data indicated a significant

difference at 60, 300 and 600 µg/mL. The test material was therefore considered to be clastogenic in the activation system. The types of chromosomal aberrations included primary chromatid breaks and rearrangements. Of interest is the observation that at 600 µg/mL, cells in anaphase and cells in metaphase are both present, suggesting that the presence of TGHP interfered with the metastatic activity of Colcemid. Furthermore, while not quantitated, anaphase bridges were observed among these cells, supporting the clastogenic interpretation. The positive control, cyclophosphamide at 25 µg/mL, was clastogenic.

g. Mouse Lymphoma Mutation Assay.

(1) The initial toxicity test conducted on TGHP indicated 100 percent toxicity through 1000 µg/mL for the nonactivated cultures and at 10,000 µg/mL for the S-9 activated cultures. Therefore, the TGHP was tested, in the mutagenesis assay, over a range of concentrations from 200 to 2.7 µg/mL for the nonactivated and from 10,000 to 134 µg/mL for the S-9 activated cultures.

(2) None of the nonactivated or activated cultures that were cloned exhibited a mutant frequency that was at least twice the mean mutant frequency of the solvent control. The Total Growths of the nonactivated cultures ranged from 27 to 104 percent and 32 to 100 percent for the S-9 activated cultures. A dose-dependent response was not noted in either culture.

(3) The results indicated that, under the conditions of these mutagenesis tests, TGHP was negative in both the presence and absence of exogenous metabolic activation.

h. Unscheduled DNA Synthesis Assay (UDS).

(1) The UDS assay was initiated with ten treatments of TGHP ranging from 0.5 to 1000 µg/mL in an attempt to obtain a good range of toxicities for analysis. The UDS was measured at concentrations of the test compound between 10 and 750 µg/mL. The UDS was not measured at 0.5, 1, and 5 µg/mL because enough higher concentrations in the assay were available for evaluation. The net grain/nucleus (NG) counts were negative for all concentrations of the vehicle control and the medium control, yielding mean values ranging from -13.9 to -11.0 NG at a level of 1 percent of cells in repair (percent IR), in contrast to the strong positive response produced by 2-AAF (33.2 NG, 96 percent IR). Concentrations of TGHP, ranging from 10.0 to 750 µg/mL, all

yielded negative mean net grains/nucleus values ranging from -10.2 to -14.2 NG with percent in repair values ranging from 0 to 6 percent IR. A toxic response was observed at the highest concentration of 1,000 µg/mL.

(2) In the assay a test article was considered unequivocally positive if the mean grain count for any dose group was greater than 5 NG. Therefore, based on the criteria for a positive response, TGHP was negative in the *in vitro* rat hepatocyte DNA repair assay.

9. DISCUSSION.

a. The purpose of these studies was to determine the relative acute toxicity of TGHP, the active ingredient in the Army's water purification tablets. Data from these studies are used to recommend and initiate additional acute and subchronic studies to satisfy the EPA's data call-in for toxicological data on antimicrobial pesticide active ingredients.

b. A review of the results from these acute studies show that TGHP is moderately toxic by ingestion when prepared as a aqueous slurry. The compound has no potential for causing sensitization. However, TGHP did produced mild but reversible primary skin irritation, and the technical grade powder did cause severe and nonreversible injury to the eye.

c. The TGHP did not exhibit mutagenic activity in three of the four mutagenicity assays performed. In the Chromosome Aberration Assay, TGHP did produce a significant increase in chromosomal aberrations in CHO cells in the presence, but not in the absence, of metabolic activation. This single positive finding in the Chromosome Aberration Assay, in the presence of metabolic activation, is not considered sufficient to classify TGHP a potential human-cell mutagen.

d. Additional studies involving TGHP are to be conducted at this Agency. These studies include preliminary 14-day and 90-day feeding studies, and teratology studies in rats and rabbits. Additional studies are being done to determine if TGHP will cause dominant lethal effects in a mammalian test system. Preliminary avian wildlife hazard evaluation studies are also in progress.

10. RECOMMENDATIONS. The following recommendations are based on professional scientific judgement.

a. Continue acute and subchronic toxicity studies listed in paragraph 9d in support of the requirement to provide data to EPA for the reregistration of Army's Water Purification Tablets.

b. Enforce the use of protective eyewear during operations involving the use or mixing of the technical grade powder because of severe eye irritation potential.

JOSEPH A. MACKO, JR.
Biologist
Toxicology Division

APPROVED:

MAURICE H. WEEKS
Chief, Toxicology Division

APPENDIX A

REFERENCES

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Phase 1, Toxicological Study No. 75-51-0742-91, Jan 88 - Aug 91

APPENDIX B

TECHNICAL COMPLIANCE STATEMENT

I certify as the Technical Study Director that the referenced study was conducted under the following compliance conditions:

a. These studies were conducted in accordance with approved Standing Operating Procedures developed by the Toxicology Division, USAEHA and approved by me and the Laboratory Animal Use Review Committee.

b. Toxicological and chemical raw data collected during these studies are archieved in Building 1570, in the USAEHA Library, Toxicology Storage Room, under Project Number 75-51-0742-91.

MAURICE H. WEEKS
TECHNICAL STUDY DIRECTOR

APPENDIX C

ANALYTICAL QUALITY ASSURANCE

The Analytical Quality Assurance Office certifies the following:

a. These studies were conducted in accordance with Standing Operating Procedures developed by the Toxicology Division, USAEHA.

b. Facilities were inspected during its operational phase to ensure compliance with paragraph a above.

c. The information presented in this report accurately reflects the raw data generated during the course of conducting these studies.

TIMOTHY L. FISHER
Chief, Analytical Quality
Assurance Office

APPENDIX D

SCALE FOR SCORING SKIN LESIONS
DRAIZE SYSTEM

1.	ERYTHEMA AND ESCHAR FORMATION.	
	a. No erythema	0
	b. Very slight erythema (barely perceptible)	1
	c. Well defined erythema	2
	d. Moderate-to-severe erythema	3
	e. Severe erythema ("beet" redness to slight eschar formation injuries in depth)	4
	f. Possible total erythema score	4
2.	EDEMA FORMATION.	
	a. No edema	0
	b. Very slight edema (barely perceptible)	1
	c. Slight edema (edges of area well defined by defined raising)	2
	d. Moderate edema (edges raised approximately 1 mm)	3
	e. Severe edema (raised more than 1 mm and extending beyond area of exposure)	4
	f. Possible total edema score	4
3.	POSSIBLE TOTAL SCORE FOR PRIMARY IRRITATION.	8

APPENDIX E

DEFINITION OF CATEGORIES OF COMPOUNDS BEING CONSIDERED FOR ACUTE SKIN APPLICATION

CATEGORY I. Compound producing no primary irritation of the intact skin or no greater than mild primary irritation of the skin surrounding an abrasion. (INTERPRETATION: No restriction for acute application to the human skin.)

CATEGORY II. Compounds producing mild primary irritation of the intact skin and the skin surrounding an abrasion. (INTERPRETATION: Should be used only on human skin found by examination to have no abrasions or may be used as a clothing impregnant.)

CATEGORY III. Compounds producing moderate primary irritation of the intact skin and the skin surrounding an abrasion. (INTERPRETATION: Should not be used directly on the skin without a prophetic patch test having been conducted on humans to determine irritation potential to human skin. May be used without patch testing, with extreme caution, as clothing impregnants. Compound should be resubmitted in the form and at the intended use concentration so that its irritation potential can be reexamined using other test techniques on animals.)

CATEGORY IV. Compounds producing moderate to severe primary irritation of the intact skin and of the skin surrounding an abrasion and, in addition, producing necrosis, vesiculation and/or eschars. (INTERPRETATION: Should be resubmitted for testing in the form and at the intended use concentration. Upon resubmission, its irritation potential will be reexamined using other patch test techniques on animals, prior to possible prophetic patch testing in humans, at concentrations which have been shown not to produce primary irritation in animals.)

CATEGORY V. Compounds impossible to classify because of staining of the skin or other masking effects owing to physical properties of the compound or compounds producing necrosis, vesiculation, or eschars. (INTERPRETATION: Not suited for use in humans.)

APPENDIX F

ACUTE SKIN EFFECTS NEW ZEALAND WHITE RABBITS

COMPOUND: Tetraglycine Hydroperiodide — **USAEHA STUDY NO.** 75-50-0742-88

PRIMARY SKIN EFFECT NEW ZEALAND WHITE RABBITS	TOXICITY CATEGORY* II	CONDITIONS: Single 24-hour applications of 0.5 gm TGHP paste (power moistened with water) per skin application site. Occluded.

	Time of Observation	Response Rabbit No.							
	Hours	31	32	33	34	35	36	Mean Score	Comments
Erythema & Eschar									
									EPA CATEGORY IV
Intact Skin	24		0		0		0	0.00	
Intact Skin	72		2		0		0	0.66	
Intact Skin	7 days		0		0		0	0.00	
Abraded Skin	24	0		0		0		0.00	
Abraded Skin	72	2		0		0		0.66	
Abraded Skin	7 days	0		0		0		0.00	
						Subtotal		1.32	
Edema Formation									
Intact Skin	24		2		2		2	2.00	
Intact Skin	72		2		1		0	1.00	
Intact Skin	7 days		0		0		0	0.00	
Abraded Skin	24	1		1		1		1.00	
Abraded Skin	72	2		0		0		0.66	
Abraded Skin	7 days	0		0		0		0.00	
						Subtotal		4.66	
						Total		5.98	

*40 CFR 162

Skin reactions are evaluated using Draize scoring system. Draize, J.H., Woodward, G. and Calvary, H.O., Methods for the Study of Irritation of Toxicity of Substances Applied Topically to the Skin and Mucous Membranes, J. Pharmacol and Exp Therap., 82: 777,290,944.

APPENDIX G

SCALE FOR SCORING OCULAR LESIONS
DRAIZE SYSTEM

1. Cornea:

   a. Opacity: - Degree of Density

No opacification	0
Scattered or diffuse area; details of iris clearly visible	1
Easily discernible translucent areas; details of iris slightly obscured	2
Opalescent areas; no details of iris visible, size of pupil barely discernible	3
Opaque; iris invisible	4

   b. Area of Cornea Involved:

One Quarter (or less) but not zero	1
Greater than one quarter but less than one-half	2
Greater than one-half but less than three quarters	3
Greater than three quarters up whole area	4

   Score = (a) x (b) x 5. Total maximum score = 80

2. Iris:

   a. Values:

Normal	0
Folds above normal, congestion, swelling, circumcorneal injection (any one or all of these or combination of any thereof), iris still reacting to light (sluggish reaction is positive	1
No reaction to light, hemorrhage; gross destruction (any one of these)	2

   Score = (a) x 5. Total maximum score = 10

3. Conjunctivae:

a. Redness: (Refers to palpebral and bulbar conjunctivae excluding cornea and iris).

Blood vessels normal	0
Some vessels definitely injected above normal	1
More diffuse, deeper crimson red, individual vessels not easily discernible	2
Diffuse, beefy red	3

b. Chemosis:

No swelling	0
Any swelling above normal (includes nictitating membrane	1
Obvious swelling with partial eversion of the lids	2
Swelling with lids half closed	3
Swelling with lids half closed to completely closed	4

c. Discharge:

No discharge	0
Any amount different from normal (does not include small amount observed in inner canthus of normal animals)	1
Discharge with moistening of the lids and hair adjacent to the lids	2
Discharge with moistening of the lids and considerable area around the eye	3

Score equals (a + b + c) x 2. Total maximum = 20

APPENDIX H

DEFINITION OF CATEGORIES OF EYE EFFECTS

1. Category A. Compounds noninjurious to the eye. Eye injury score limits: 0-10 (individual conjunctival score for chemosis, redness or discharge not to exceed 1).
   Interpretation - Irritation of human eyes is not expected if the substance should accidentally get into the eyes, provided it is washed out as soon as possible.

2. Category B. Compounds producing mild injury to the cornea. Eye injury score limits: 10-20 (individual conjunctival score for chemosis, redness or discharge not to exceed 1).
   Interpretation - To be used with caution around the eyes.

3. Category C. Compounds producing mild injury to the cornea and, in addition, some injury to the conjunctivae.
   Eye injury score limits: 5-30 (individual conjunctival score for chemosis, redness or discharge exceeds 1).
   Interpretation - To be used with caution around the eyes and mucosa (nose and mouth).

4. Category D. Compounds producing moderate injury to the cornea. Eye injury score limits: >20-50 (individual conjunctival score for chemosis, redness, or discharge not to exceed 1).
   Interpretation - To be used with extreme caution around the eyes. Keep away from ocular area.

5. Category E. Compounds producing moderate injury to the cornea and, in addition, producing some injury to the conjunctivae. Eye injury score limits: >20-50 (individual conjunctival score for chemosis, redness, or discharge exceeds 1).
   Interpretation - To be used with extreme caution around the eyes and mucosa (e.g., nose and mouth). Keep away from ocular areas.

6. Category F. Compounds producing severe injury to the cornea and conjunctivae.
   Eye injury score limits: >50.
   Interpretation - To be used with extreme caution; recommended that use be restricted to areas other than the face.

APPENDIX I

ACUTE EYE EFFECTS NEW ZEALAND WHITE RABBITS

INDIVIDUAL ANIMAL SCORES

COMPOUND: TETRAGLYCINE HYDROPERIODIDE

TIME	STRUCTURE	RABBIT NUMBER / SCORE						
		376	377	378	379	380	381	MEAN
24 HR	Cornea	40	40	60	80	80	40	56.7
	Iris	5	5	5	10	10	5	6.7
	Conjunctiva	16	12	16	16	16	14	15.0
48 HR	Cornea	60	40	40	60	40	20	43.3
	Iris	5	5	5	5	0	5	4.2
	Conjunctiva	10	14	14	12	10	12	12.0
72 HR	Cornea	40	40	40	40	40	20	36.7
	Iris	5	5	10	5	5	5	5.8
	Conjunctiva	6	10	10	10	10	10	9.3
7 DAY	Cornea	40	40	60	60	40	40	46.7
	Iris	5	5	5	5	5	5	5.0
	Conjunctiva	6	10	6	12	6	8	8.0
14 DAY	Cornea	5*	0	10*	45*	0	10	11.7
	Iris	0	0	5	5	5	0	2.5
	Conjunctiva	0	6	0	12	4	6	4.7
21 DAY	Cornea	5*	0	5*	20*	0	5	5.8
	Iris	0	0	5	5	0	0	1.7
	Conjunctiva	0	4	0	4	4	4	8.0

* Neovascularization.

TGHP (solid) was corrosive to rabbit eyes, causing injury to the conjunctiva and cornea which persisted through 21 days. USEPA Category - I